



ISSN: 2456-2912

NAAS Rating (2025): 4.61

VET 2025; 10(10): 105-110

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www.veterinarypaper.com

Received: 22-07-2025

Accepted: 29-08-2025

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Evaluating Neem, Moringa and coconut oil in calf diets: Productivity gains with enteric methane abatement

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DOI: <https://www.doi.org/10.22271/veterinary.2025.v10.i10b.2614>

Abstract

This study assessed how growing crossbred calves' growth performance, nutrient utilization, and enteric methane abatement were affected by the phytochemical-rich feed additives Neem (*Azadirachta indica*) leaf powder, *Moringa oleifera* leaf powder, and coconut oil. Twenty calves (6-12 months) were divided into four groups at random (N=5/treatment) and fed four different diets for 120 days: coconut oil at 2% of DMI (T₃), Neem leaf powder at 2% of DMI (T₁), Moringa leaf powder at 2% of DMI (T₂), and control (T₀). A standard concentration and seasonal green/dry fodders were incorporated in diets that complied with ICAR (2013) guidelines. A 7-day digestibility trial was carried out after 120 days, and Ellis *et al.* (2007) were used to estimate methane emissions from intake. At days 30, 60, and 120, blood biochemical indicators (glucose, total protein, albumin, globulin, and A:G) were evaluated; modified Sheather's sugar flotation was used to evaluate fecal parasitology. Without having a negative impact on digestibility, moringa dramatically increased average daily gain and DMI. While coconut oil shown methane-lowering patterns characteristic of lipid supplementation, both Moringa and Neem decreased the estimated methane output in comparison to the control. Neem had an anthelmintic effect by lowering the number of fecal eggs. Better growth was accompanied by slight improvements in serum biochemistry, which stayed within physiological ranges. A sustainable way to increase calf productivity while reducing enteric methane is to use phytochemical additives, especially Moringa at 2% DMI. Methane reductions should be verified by actual measurement, and inclusion rates should be optimized in future work.

Keywords: Phytochemicals, *Moringa oleifera*, Neem, coconut oil, calves, digestibility, methane mitigation, growth performance, anthelmintic

Introduction

Ruminant livestock produce enteric methane, which accounts for almost 30% of all anthropogenic methane worldwide and is a significant contributor to agricultural greenhouse gas emissions (Gerber *et al.*, 2013; Hristov *et al.*, 2013) ^[1, 13]. Therefore, a major emphasis of climate change mitigation efforts is livestock systems, especially cattle (FAO, 2019; IPCC, 2021) ^[2, 3]. Simultaneously, smallholder and commercial systems must improve the development and feed efficiency of young animals, particularly in emerging nations where inefficient calf raising limits future output (Thornton & Herrero, 2010). Therefore, it is very beneficial to implement interventions that can both improve animal performance and lower methane emissions.

Because it may be implemented at the farm level and included into current rations, dietary modification with feed additives is one of the most feasible mitigation options (Beauchemin *et al.*, 2008; Knapp *et al.*, 2014) ^[5, 6]. Along with lipid supplementation, phytochemical feed additives that contain secondary metabolites like tannins, saponins, and essential oils have demonstrated promise in changing rumen fermentation and lowering methane production (Patra & Saxena, 2010; Jayanegara *et al.*, 2012) ^[7, 8]. It is well known that tannins and saponins reduce the number of protozoa and reroute fermentation pathways to propionate, which decreases the amount of hydrogen available to methanogens (Waghorn, 2008; Goel & Makkar, 2012) ^[9, 10]. According to Benchaar *et al.* (2008) ^[11], essential oils have antibacterial capabilities against particular microbial species.

Although greater quantities of lipids may hinder the digestion of fiber, lipids, especially medium-chain fatty acids (MCFA), can reduce protozoa and methanogens (Machmüller *et al.*, 2003; Hristov *et al.*, 2013) [12, 13].

Because of its high crude protein content, balanced amino acid profile, vitamins, minerals, and bioactive substances like flavonoids and phenolics, *Moringa oleifera* leaves is particularly promising among phytogetic possibilities (Makkar & Becker, 1997; Anwar *et al.*, 2007) [14, 15]. *Moringa* leaves have been shown in a number of *in vivo* ruminant studies to enhance feed intake, digestibility, and weight growth (Nouman *et al.*, 2014; Ayssiweide *et al.*, 2011) [16, 17]. Its phytochemicals may also have a positive effect on rumen fermentation and lower gas production (Soliva *et al.*, 2005) [18]. Due to its immunomodulatory, antibacterial, and antiparasitic qualities, *Azadirachta indica*, or Neem, has long been utilized in ethnoveterinary medicine (Biswas *et al.*, 2002) [19]. Due to the impact of tannins and limonoids on rumen bacteria and gastrointestinal parasites, neem leaf supplementation has shown increases in animal performance and anthelmintic effects (Egualde *et al.*, 2011) [20], (Kumar *et al.*, 2016) [21]. Neem is a useful supplement for sustainable livestock production because of its dual benefits of enhancing animal health and perhaps reducing methane emissions.

Lauric acid, which has potent antiprotozoal and antimethanogenic qualities, is one of the several MCFA found in coconut oil (Machmüller *et al.*, 2003; Dohme *et al.*, 1999) [12, 22]. It has been demonstrated that adding coconut oil to

ruminant diets lowers methane output; however, the extent of this decrease varies depending on the dose and the initial diet composition (Hristov *et al.*, 2013; Maia *et al.*, 2007) [13, 23].

Few studies have directly examined locally accessible phytogetic supplements and a lipid source under the same circumstances in developing calves, despite mounting evidence that phytochemicals and lipids can be used as methane mitigation techniques. In order to assess the effects of Neem leaf powder, *Moringa oleifera* leaf powder, and coconut oil (each at 2% of DMI) on growth performance, nutrient utilization, fecal parasitology, serum biochemistry, and estimated enteric methane emissions in crossbred calves, the current study was conducted at MPKV, Rahuri, Maharashtra. With no negative effects on digestibility or health, we predicted that supplementing with these locally accessible feed additives would increase calf productivity while reducing methane output.

Materials and Methods

Each calf was fed separately and kept in a shed with good ventilation. According to ICAR (2013) [24] feeding guidelines, the animals were given a concentrate combination and roughages (dry and green fodder) for a daily body weight increase of 500g. Two times, at 11:00 and 15:00, fresh and clean drinking water was made available. Calves were vaccinated and dewormed in accordance with routine procedure prior to the experimental trial commencing (Table 1).

Table 1: Experimental design, treatments, and measurements in growing calves at MPKV, Rahuri

Parameter	Details
Location	MPKV, Rahuri, Maharashtra, India
Animal Ethics	Maintained as per institutional animal ethics guidelines
Animals	20 crossbred calves, 6–12 months old, mixed sexes
Experimental Design	Randomized block design based on initial body weight; 4 treatment groups (N=5 each)
Treatment Groups	T ₀ (Control): No additive T ₁ : Neem leaf powder @ 2% DMI T ₂ : <i>Moringa oleifera</i> leaf powder @ 2% DMI T ₃ : Coconut oil @ 2% DMI
Feeding Duration	120 days feeding trial + 7-day digestibility trial
Basal Diet	Concentrate mixture + seasonal green and dry fodders, meeting ICAR (2013) [24] nutrient standards
Measurements-Growth	Body weight recorded at 0, 30, 60, 90, and 120 days; calculation of average daily gain (ADG)
Measurements-Feed	Daily feed offered, refusals, and intake (kg/d); DMI expressed as% of body weight
Digestibility Trial	Total faecal collection for 7 days post 120-day feeding; chemical composition analyzed for proximate and fiber fractions (AOAC, 2012; Van Soest <i>et al.</i> , 1991) [25, 26]
Methane estimation	Estimated from intake using Ellis <i>et al.</i> (2007): CH_4 (MJ/d) = $2.70 \pm 1.38 + (1.16 \pm 0.271) \times DMI$ (kg/d) - $(15.8 \pm 6.86) \times EE$ (kg/d).
Fecal parasitology	Modified Sheather's sugar flotation for egg/oocyst counts (eggs per gram, EPG).

Table 2: Methods used for laboratory analyses of feed samples

Parameter	Method Used
Dry Matter (DM)	Oven drying
Crude Protein (CP)	Kjeldahl method (N × 6.25)
Ether Extract (EE)	Soxhlet extraction
Ash	Muffle furnace incineration
Neutral Detergent Fiber (NDF)	Van Soest method
Acid Detergent Fiber (ADF)	Van Soest method

Analysis of statistics

Treatment as a fixed effect in a completely randomized design (CRD). Repeated measurements ANOVA for longitudinal outcomes; ANOVA for treatment means. Trends 0.05 ≤ *p* < 0.10; significance at *p* < 0.05. Tukey's HSD is used for post-hoc comparisons where appropriate. Mean ± SE is the reported data.

Results and Discussion

Animal performance

Initial BW and allocation were comparable across groups. Body weight gain: T₂ (*Moringa*) showed the highest ADG (Table 2), significantly exceeding T₀; T₁ (Neem) was intermediate and generally higher than T₀; T₃ (coconut oil) showed modest gains, similar or slightly above control. ADG:

$T_2 > T_1 \geq T_3 \geq T_0$ ($p < 0.05$ for T_2 vs T_0 ; other pairwise differences as trends or significant depending on time point). *Moringa oleifera* leaf powder at 2% of DMI significantly improved growth in growing calves, accompanied by higher intake and preserved digestibility. The high crude protein content and favorable profile of vitamins, minerals, and bioactive compounds likely supported both rumen microbial efficiency and host metabolism. The methane-abating effect with *Moringa* aligns with literature attributing reductions to polyphenols, saponins, and shifts in fermentation towards propionate.

Table 3: Impact of giving feed additives including phytochemicals on developing calves' average daily gain (ADG, gm)

Parameter	T ₀	T ₁	T ₂	T ₃	SEM	C.D.
Day 15	306.0±55 ^c	458.0±21 ^{ab}	502.0±31 ^a	377.0±41 ^{bc}	39.16	109.6
Day 30	310.0±19 ^b	512.0±15 ^a	536.0±48 ^a	394.0±57 ^b	38.85	106.8
Day 45	362.0±20	524.0±24	549.0±33	468.0±112	60.28	173.6
Day 60	393.0±6 ^c	548.0±20 ^{ab}	639.0±30 ^a	513.0±62 ^b	35.97	104.3
Day 75	434.0±32 ^c	569.0±33 ^{ab}	651.0±34 ^a	538.0±24 ^b	31.00	91.5
Day 90	508.0±49 ^c	618.0±21 ^{ab}	660.0±18 ^a	517.0±38 ^{bc}	33.96	100.9
Day 105	514.0±23 ^c	585.0±24 ^b	678.0±18 ^a	547.0±14 ^{bc}	20.21	56.0
Day 120	522.0±16 ^c	640.0±28 ^{ab}	748.0±53 ^a	579.0±38 ^{bc}	36.52	109.6
Overall Mean	419.0±28 ^c	557.0±23 ^{ab}	620.0±33 ^a	492.0±48 ^{bc}	36.99	106.9

^{abc}Means without a common superscript vary within a column ($p < 0.05$). CD at 5%; Control, T₀; T₁, 2% DM basis powdered neem leaves; T₂, 2% DM basis powdered *Moringa oleifera*; T₃, 2% DM basis coconut oil; T, treatment; SEM, standard error of mean; P, probability.

Dry Matter Intake (kg/d)

Increased in T₂ vs T₀ ($p < 0.05$); T₁ typically comparable or slightly improved; T₃ similar to control. DMI (% BW): Reflected the kg/d pattern; T₂ greater than T₀ ($p < 0.05$). FCR: Improved (lower) in T₂ versus T₀; T₁ modest improvement; T₃ neutral to slightly improved (Table 3).

T₂ (*Moringa oleifera*) had a greater total DMI, which could be because of its superior growth rate and improved palatability. The other researcher's findings corroborated the facts (Abd

Because *Moringa* leaves, fresh pods, seeds, and roots contain more vital nutrients, both people and animals are using them more and more (CSIR 1962; Hartwell 1971) [28, 29]. As noted by Bose (1980) [31] and El-Badawi *et al.* (2014) [32], Ahmad *et al.* (2017) [30] also proposed that feeding suckling buffalo calves dried *Moringa oleifera* leaves (DMOL) at levels of 5, 10, and 15% might act as a natural growth enhancer. The antibacterial qualities of *Moringa* (Fahey, 2005) [35] and the high nutritional content of DMOL (Sarwatt, 2004; Kakengi, 2005) [33, 34] may be responsible for the improvement in body weight increase.

El-Aal, 2000) [36]. The addition of 0.75 and 1.0 mg/ml of *Moringa oleifera* aqueous methanol extract significantly ($p < 0.05$) reduced the apparent dry matter digestibility by 7.94 and 13.53 percent compared to control, according to Alexander *et al.* (2008) [37]. Additionally, they discovered that there was no significant difference in actual dry matter digestibility between the control and *Moringa oleifera* aqueous methanol extract.

Table 4: Impact of providing feed additives including phytochemicals on developing calves' weekly dry matter intake (DMI, kg)

Parameters	T ₀	T ₁	T ₂	T ₃	SEM	C.D.
Day 0	1.76±0.06	1.77±0.09	1.79±0.03	1.75±0.05	0.06	NS
Day 15	1.88±0.05	2.06±0.14	2.14±0.09	1.91±0.06	0.09	0.25
Day 30	1.96±0.04 ^c	2.28±0.10 ^{ab}	2.42±0.03 ^a	2.16±0.07 ^b	0.06	0.17
Day 45	2.18±0.07 ^c	2.49±0.07 ^b	2.68±0.05 ^a	2.36±0.06 ^{bc}	0.06	0.17
Day 60	2.40±0.05 ^c	2.73±0.11 ^b	3.04±0.03 ^a	2.62±0.08 ^{bc}	0.07	0.21
Day 75	2.62±0.06 ^c	3.06±0.07 ^b	3.31±0.04 ^a	2.87±0.08 ^b	0.06	0.18
Day 90	2.90±0.03 ^c	3.31±0.06 ^b	3.58±0.05 ^a	3.15±0.08 ^b	0.06	0.17
Day 105	3.21±0.03 ^d	3.65±0.06 ^b	3.92±0.04 ^a	3.42±0.05 ^c	0.04	0.12
Day 120	3.33±0.07 ^c	3.78±0.04 ^b	4.41±0.06 ^a	3.62±0.11 ^b	0.08	0.23
Overall Mean	2.47±0.05 ^c	2.79±0.08 ^b	3.03±0.05 ^a	2.66±0.07 ^b	0.06	0.19

^{abc}Means without a common superscript vary within a column ($p < 0.05$). CD at 5%; Control, T₀; T₁, 2% DM basis powdered Neem leaves; T₂, 2% DM basis powdered *Moringa oleifera*; T₃, 2% DM basis coconut oil; T, treatment; SEM, standard error of mean; P, probability.

The current study's findings concur with those of Aharwal *et al.* (2018) [38] and Ahmad *et al.* (2017) [30], who found a substantial change in FCR when buffalo calves' diets were supplemented with dried *Moringa oleifera* leaves (DMOL). This might be the result of experimental calves meeting their recommended needs and receiving their recommended nutrient allowances (A.P.R.I., 1997) [39]. Jiwuba *et al.* (2016) [41] and Khalel *et al.* (2014) [40] similarly noted a noteworthy variation in FCR when *Moringa oleifera* leaves were added to goats' diets.

Methane estimation

Estimated CH₄ output (MJ/d and g/kg DMI) decreased in T₂ and T₁ relative to T₀; T₃ also decreased due to EE effect but

balanced by intake. Relative ranking: T₂ ≈ T₃ ≈ T₁ < T₀ for CH₄ yield (g/kg DMI) with significant reductions for T₂ and T₁ ($p < 0.05$). Coconut oil showed expected reduction; often trend-level depending on intake.

Tables 4 and 5 show the methane emission results and group comparisons. Using the Ellis *et al.* (2007) equation, the average methane emission (MJ/d/animal) was 5.27±0.08 (T₀), 4.86±0.11 (T₁), 4.82±0.05 (T₂), and 4.15±0.08 (T₃). Methane production decreased by 21.26% in the coconut oil (T₃), 8.53% in the *Moringa oleifera* (T₂), and 7.77% in the Neem leaf powder (T₁) groups as compared to the control (T₀). Statistical analysis revealed a highly significant ($p < 0.05$) decrease among treatments, with the control group exhibiting the greatest emission and coconut oil (T₃) the lowest.

Table 5: Methane (MJ/Day) gas emissions in growing calves as a result of feeding phytochemicals containing feed additives

Parameters	T ₀	T ₁	T ₂	T ₃	SEM	C.D.
Day 1	5.21±0.10 ^a	4.80±0.10 ^b	4.82±0.04 ^b	4.14±0.07 ^c	0.07	0.20
Day 2	5.34±0.07 ^a	4.84±0.10 ^b	4.83±0.06 ^b	4.15±0.06 ^c	0.08	0.22
Day 3	5.22±0.12 ^a	4.91±0.11 ^b	4.80±0.08 ^b	4.11±0.05 ^c	0.08	0.23
Day 4	5.35±0.06 ^a	4.78±0.13 ^b	4.85±0.03 ^b	4.09±0.10 ^c	0.08	0.23
Day 5	5.36±0.07 ^a	4.93±0.11 ^b	4.81±0.05 ^b	4.11±0.11 ^c	0.09	0.27
Day 6	5.18±0.08 ^a	4.95±0.13 ^b	4.82±0.06 ^b	4.21±0.12 ^c	0.08	0.24
Day 7	5.26±0.08 ^a	4.81±0.12 ^b	4.83±0.03 ^b	4.24±0.06 ^c	0.08	0.22
Overall Mean	5.27±0.08 ^a	4.86±0.11 ^b	4.82±0.05 ^b	4.15±0.08 ^c	0.08	0.25

^{abc}Means without a common superscript vary within a column ($p<0.05$). CD at 5%; Control, T₀; T₁, 2% DM basis powdered Neem leaves; T₂, 2% DM basis powdered *Moringa oleifera*; T₃, 2% DM basis coconut oil; T, treatment; SEM, standard error of mean; P, probability.

The results are consistent with those of Dong *et al.* (1997), who found that coconut oil decreased CH₄ by 85% in concentrate diets and 59% in grass hay diets. In a similar vein, Machmuller and Kreuzer (1999) found that supplementing sheep with 3.5-7% coconut oil reduced methane by 28-73% without impairing their ability to absorb nutrients. According to Dohme *et al.* (2001) [45], medium-chain fatty acids (MCFA) in coconut oil are responsible for the greater methane

suppression observed when lauric acid is combined with it as opposed to myristic acid.

As anticipated, methane-lowering properties were demonstrated by coconut oil, which is high in lauric acid, a medium-chain fatty acid. Negative effects on fiber digestibility were negligible at 2% DMI, indicating that this relatively modest inclusion strikes a compromise between rumen function maintenance and methane mitigation.

Table 6: Methane gas (MJ/Day) emissions in developing calves as a result of eating phytochemicals containing feed additives

Parameters	T ₀	T ₁	T ₂	T ₃	SEM	C.D.
DMI kg/day	3.638±0.06 ^b	3.693±0.06 ^b	3.918±0.03 ^a	3.638±0.1 ^b	0.065	0.18
EELI kg/day	0.104±1.06 ^d	0.135±1.47 ^c	0.152±1.85 ^b	0.176±1.5 ^a	1.53	4.21
*CH ₄ (MJ/Day/Calve)	5.28±0.08 ^a	4.85±0.11 ^b	4.83±0.05 ^b	4.14±0.9 ^c	0.08	0.23
% Reduction	--	7.77	8.53	21.26	--	--

*CH₄ computation using Ellis *et al.* 2007's regression equation

^{abcd}Means without a common superscript vary within a column ($p<0.05$). CD at 5%; Control, T₀; T₁, 2% DM basis powdered Neem leaves; T₂, 2% DM basis powdered *Moringa oleifera*; T₃, 2% DM basis coconut oil; T, treatment; SEM, standard error of mean; P, probability.

Fecal parasitology: Impact on Nematodes and Cestodes in Calves

Neem (T₁) reduced fecal egg counts compared with T₀ ($p<0.05$), indicating an anthelmintic effect. *Moringa* (T₂) showed neutral to modest improvements; coconut oil (T₃) largely neutral. Faecal samples collected at fortnightly intervals showed almost nil egg counts across all treatments, indicating negligible infection and failure of natural infection to establish in calves. Results for roundworm egg counts are presented in Tables 4.33-4.35 (30th, 60th, and 120th day). Neem leaves powder (T₁) was found superior in reducing roundworm eggs compared to T₀, T₂, and T₃.

Neem leaf powder delivered tangible anthelmintic benefits and reduced estimated methane yield, consistent with the presence of limonoids, tannins, and other phytochemicals that can suppress protozoa and methane-associated microbial pathways. Reduced parasitic load also supports improved nutrient utilization.

Amin *et al.* (2009) [46] found that extracts of *A. indica* leaves powder, tobacco, barbados lilac, betel leaf, pineapple, jute, turmeric, garlic, dodder, and bitter gourd (100 mg/ml) were 100% effective against adult gastrointestinal parasites. These

results are consistent with their findings. Additionally, Cabardo and Portugaliza (2017) [47] showed that *Moringa oleifera* seed ethanolic and aqueous extracts had anthelmintic potential against *Haemonchus contortus* eggs and L3 larvae, with larvicidal efficacy of 56.94% and 92.50% at 7.8 mg/mL and egg hatch inhibition of 95.89% and 81.72% at 15.6 mg/mL. Secondary metabolites are probably in charge of the anthelmintic effects of the bioactive compounds found in *M. oleifera* (Wang, 2016) [48].

Neem leaf powder (T₁) was more effective than T₀, T₂, and T₃, according to tapeworm egg counts. Akbar and Ahmed (2003) [49] found that pineapple (82%) and albendazole (88%) reduced fecal egg counts more than Neem leaves (56%; $p<0.05$), with comparable outcomes on day 21. The current results corroborate those of Cabardo and Portugaliza (2017) [47], who discovered saponins in aqueous extracts of *M. oleifera* and tannins in ethanolic extracts. The anthelmintic qualities of *M. oleifera* seed bioactive components against *H. contortus* were confirmed by both extracts, which immobilized L3 larvae and hindered larval growth within eggs.

Table 7: Cumulative effect of feeding phytochemical-based feed additives on roundworms and tapeworms in growing calves

Treatment	30 th Day Roundworms	30 th Day Tapeworms	60 th Day Roundworms	60 th Day Tapeworms	120 th Day Roundworms	120 th Day Tapeworms
T ₀ (Control)	++ to +++	++ to +++	++ to +++	++ to +++	++ to +++	++ to +++
T ₁ (Neem leaf powder 2% DMI)	+ to ++	+ to ++	+ to ++	+ to ++	- to +	- to +
T ₂ (<i>Moringa leaf powder</i> 2% DMI)	++ to +++	+ to +++	+ to ++	+ to ++	- to +	+
T ₃ (Coconut oil 2% DMI)	++ to +++	+ to ++	+ to ++	+ to ++	+ to ++	+ to ++

“+++” = heavy infection (100% slide filled with eggs/worms); “++” = moderate infection; “+” = light infection; “-” = no infection observed

Conclusion

Supplementation of growing calf diets with phytochemical additives at 2% of DMI improved performance and reduced estimated enteric methane emissions without compromising animal health. *Moringa oleifera* leaf powder produced the largest gains in ADG and DMI with neutral-to-positive effects on digestibility and serum profile. Neem leaf powder reduced fecal egg counts and methane yield, supporting health and environmental outcomes. Coconut oil tended to reduce methane with minimal impact on digestibility at the tested level. Phytochemicals, particularly *Moringa* at 2% DMI, are promising for integrated productivity and methane mitigation strategies in calf rearing. Validation with direct methane measurements and optimization of inclusion rates is warranted.

Conflict of Interest

Not available

Financial Support

Not available

Reference

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How to Cite This Article

Bhosale TR, Rathod KD, Munnarwar SR, Wagh SD, Lokhande AT. Evaluating Neem, Moringa and coconut oil in calf diets: Productivity gains with enteric methane abatement. *International Journal of Veterinary Sciences and Animal Husbandry*. 2025;10(10):105-110.

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